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OPP/HED/RAB3 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction Study - Rat- OPPTS 870.3800 (§83-4)

DP BARCODE:D273332 SUBMISSION CODE:S593773
P.C. CODE:129058

TEST MATERIAL (PURITY):Tetrakis (hydroxymethyl) phosphonium sulphate [THPS]; purity 78.0%

SYNONYMS: Tolcide®

CITATION: Wood, E., Brooks, P.N., Doleman, N (1999). THPS: Oral Gavage Two Generation Reproduction Study in the Rat. Study performed by Safepharm Laboratories Linited, United Kingdom. Project number 1169/001. MRID *45320801*. Unpublished

SPONSOR(s): Rhodia Inc., Glen Allen, VA and Rhodia Consumer Specialties Limited, United Kingdom.

EXECUTIVE SUMMARY:

In a 2-generation reproduction study (MRID 45320801) THPS 78.0% a.i.was administered to 32 male and female Sprague-Dawley rats at doses of 0, 1.0, 7.5, or 15.0 mg/kg/day by gavage. Following ten weeks of treatment, the groups of 32 male and female rats were paired to produce the F1 litters. At weaning, 32 males and females were selected at random to form the parental F1 generation. Treatment of F1 parents continued for 11 weeks after which they were paired to produce the F2 litters. Estrous cycling of parental females was assessed prior to mating in both generations. Pups were observed for clinical signs, and litter size and pup body weight were recorded on specific days post-partum. During lactation offspring were observed for intra-litter onset and duration of physical development landmarks, Post-weaning sexual development was assessed for selected F1 males and females. Post- mortem macroscopic examinations were performed on all adults and offspring, including decedents. Reproductive and target organs were preserved in fixative for all adult P and F1 animals. Semen samples were collected from all P and F1 males at necropsy for examination. Histopathology was performed on potential target organs of selected P-F1 offspring, F1-F2 offspring and F1 adults. Oocyte numbers were examined in selected control and high dose females.

In parental animals, there were no significant effects of test article administration on body weight, food consumption, clinical signs, estrous cycling, or sperm parameters in both generations. Organ weights were affected at the 15 mg/kg/day dose level, and included an increase in liver weight for P generation females and F1 generation males and females. The increase in liver weight corresponded with histopathological alterations at the 15 mg/kg/day dose level, which included periportal hepatocyte enlargement, periportal hepatocyte vacuolation, bile duct proliferation, and focal hepatocyte necrosis. These effects were observed also at the 7.5 mg/kg/day dose and were considered treatment-related, as there was a dose-response between the 7.5 and 15.0 mg/kg/day dose levels.
Reproductive performance and fertility were not significantly affected in parental animals of either generation. Although viability index was in the range of 70-80%, this value is not atypical for the rat.

Offspring measurements were also not significantly affected by treatment with THPS at any dose level, including litter size, pup weight, reflexological assessment, and macroscopic pathology. Organ weights of the brain and spleen and thymus, which are required by the 870.3800 guideline, were not provided in this report, so it is unknown what effect if any THPS may have had on these organ weights.

Based upon the results of this study, **the parental systemic toxicity NOAEL is determined to be 1 mg/kg/day, based upon the histological alterations of the liver observed in both parental generations at the 7.5 mg/kg/day dose. The reproductive toxicity NOAEL is provisionally determined to be ≥ 15 mg/kg/day, and the reproductive toxicity LOAEL is provisionally determined to be > 15 mg/kg/day, as there were no significant effects observed in offspring at any dose level tested in this study.**
Histopathological assessment of selected pup organs was claimed in the summary of the report, but no results were presented.

This reproductive study in the rat is classified **unacceptable/upgradable** and does not satisfy the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800, §83-4). Organ weight data and histopathology data in offspring should be provided in order to upgrade this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: THPS
Description: colorless liquid
Lot/Batch #: THPS V15H1
Purity: 78.0 % a.i.
CAS #: 124-64-1

2. Vehicle: distilled water

3. Test animals: Species: rat
Strain: Sprague-Dawley CD
Age at start of dosing: approximately six weeks of age
Weight at start of dosing:
(P) Males: 188-229g; Females: 131-176g
(F_1) Males: 62-111g Females: 56-95g
Source: Charles River UK, Margate, Kent
Housing: Air-conditioned animal rooms with at least 15 air changes per hour. On arrival and through maturation periods for P and F1 adults, rats were housed 4/cage/sex in polypropylene cages with stainless steel grid floors and tops suspended over paper-lined polypropylene trays. During mating periods, animals were transferred to similar cages with one male and one female per cage.
Diet: Rat and Mouse SQC Expanded Diet No.3 ad libitum
Water: tap water ad libitum
Environmental conditions:
Temperature: 19-23 °C
Humidity: 40-70% %
Air changes: 15/hr
Photoperiod: 12 hrs dark/12 hrs light
Acclimation period (P): 14 days

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure: After the respective maturation periods, P and F1 adults were paired one male to one female for up to 13 days for the P generation and up to 17 days for the F1 generation. Cages were checked daily for the presence of ejected copulation plugs and females were also checked for the presence of vaginal copulation plugs. Presence of sperm within vaginal smears and/or presence of a vaginal plug was taken as positive evidence of mating. Males were returned to their original cages following mating, while females were separated and housed individually during gestation and lactation.

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2. Study schedule: P generation males and females were dosed for 76 days prior to mating. Vaginal smears were obtained daily for 14 days prior to mating in P generation females to evaluate estrus cycling. Following birth of the F1 litters, litter sizes were standardized at day 4 post-partum. Adult P males were killed and examined macroscopically following mating, and sperm samples also obtained. At day 21 post-partum, offspring were randomly selected for the F1 parents. Adult P generation females and unused offspring were killed and examined macroscopically. F1 parents were dosed for 82 days prior to mating and all animals were observed for external sexual development. Mating procedures were the same for the F1 parents as described for the P generation parents. Ovarian oocyte numbers were obtained from both parental female generations of the control and high dose groups following sacrifice.

3. Animal assignment: P animals were assigned to treatment groups using a randomization procedure based on stratified body weight.

TABLE 1 Animal Assignment

Test Group	Dose in Diet[a] (mg/kg/day)	Animals/group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0	32	32	32	32
Low (LDT)	1.0	32	32	32	32
Mid (MDT)	7.5	32	32	32	32
High (HDT)	15.0	32	32	32	32

aIn-life dosing was conducted between February 10 1998 and November 4 1998.

4. Dose selection rationale: According to the report (page 24) doses for this study were selected on the basis of previous toxicity data and consultation with the Sponsor's Monitoring Scientist. Previous toxicity data available to the EPA and reviewed by the Office of Pesticide Programs show NOAEL values of 5 and 10 mg/kg/day for rats and mice in subchronic studies, and NOAELs of 18 mg/kg/day and 15 mg/kg/day for maternal rats in two separate developmental toxicity studies.

5. Dosage preparation and analysis

The test material was prepared at the appropriate concentration in distilled water daily. The report included test results on stability of THPS in the dosing medium. The report also stated that THPS was unstable in aqueous media; however, results of stability testing in the report show mean percent of nominal concentrations of 96%, 100%, and 101% for the 1.0, 7.5, and 15.0 mg/kg dose level solutions. It is possible that the solutions were analyzed immediately after mixing or were analyzed after

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adjusting the pH to 12 with 1 M sodium hydroxide (page 652 of the report). Presumably, the doses were administered in distilled water right after making them up and the pH was not adjusted. Otherwise, a corrosive effect on the stomach might result as a consequence of the high pH.

Homogeneity analysis was done only by visual inspection of the dose solutions, which were stated to be homogeneous.

C. OBSERVATIONS

1. Parental animals: Observations and the schedule for those observations are summarized from the report.

Mortality and morbidity were checked twice daily during the week and once on weekends and holidays. Daily observations were made for clinical signs including immediately prior to dosing and one hour post-dosing. Body weights and food consumption were recorded once weekly during maturation and mating periods for P and F1 adults, and once weekly until termination following mating. P and F1 females with a live litter were weighed on days 1,4,7,14, and 21 post-partum. Estrous cycling was recorded for P and F1 females by vaginal smear daily for fourteen days prior to mating.

2. Litter observations: According to the report, the following litter observations (X) were made (see Table 2).

TABLE 2 F_1/F_2 Litter Observations[a]

Observation	Time of observation (lactation day)					
	Day 1	Day 4[b]	Day 4[b]	Day 7	Day 14	Day 21
Number of live pups	x	x	x	x	x	x
Pup weight	x	x	x	x	x	x
External alterations	x	x	x	x	x	x
Number of dead pups	x	x	x	x	x	x
Sex of each pup (M/F)	x	x	x	x	x	x
Reflexological assessment	x	x	x	x	x	x

a Data extracted from pages 32-34 of the report.
b Before standardization
c After standardization

Reflexological assessment included surface righting reflex, mid-air righting reflex, startle reflex, pupil reflex, and sexual development.

On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible); excess pups were killed and discarded. Litter sizes of eight or less on day 4 post-partum were not culled.

Dead pups were examined grossly for external and internal abnormalities, and a possible cause of death was determined for pups born or found dead.

3. Postmortem observations:

1) Parental animals: All adult animals found dead or in extremis were examined macroscopically for internal and external abnormalities. All surviving adults at the end of the study were killed by carbon dioxide inhalation followed by cervical dislocation and examined externally and internally for gross abnormalities.

For all P and F1 males at necropsy, a sample of semen was collected from the vas deferens of the left testis, mixed with 0.5 ml of 0.9% saline at 37 °C, and sperm motility, sperm concentration, and sperm morphology assessed.

Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera.

The following tissues (X) were prepared for microscopic examination and weighed (XX):

xx Ovaries	xx Epididymides
xx Uterus	xx Prostate
xx Vagina/cervix	xx Seminal vesicles
xx Lesions	xx Testes
xx pituitary gland	

2) Offspring: The F_1 offspring not selected as parental animals and all F_2 offspring were sacrificed and subjected to postmortem examinations in a manner similar to the adult animals, and the same tissues as listed above were examined.

D. DATA ANALYSIS

1. Statistical analyses: Gestation length, offspring sex ratio, offspring reflexological responses, group mean pre-coital length, sperm concentration, oocyte counts and organ weights relative to body weight were compared using the Kruskal Wallis non-parametric rank-sum test. Where significant differences were noted, pairwise comparison of control values against treated group values were performed using the Mann-Whitney U-test.

   Adult male and female body weight during maturation, gestation, and lactation periods, food consumption, litter size, litter weight, individual offspring body weight, physical development, and absolute organ weights were compared using Levene's test to establish homogeneity followed by one-way ANOVA. If variances were not homogenous, Dunnett's T3 Multiple Comparison Method was used. Equal variances were analyzed using Dunnett's Multiple Comparison Method.

2. Indices:

   Reproductive and viability indices: The following reproductive and viability indices were calculated from breeding and parturition records of animals in the study: pre-coital interval; gestation length;parturition index; live birth index; viability index

3. Historical control data: there were no historical control records provided with this report.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs:

In the P generation, there were a total of four deaths at 15 mg/kg/day for females, and none in males. Of the four females, one was found dead and post-mortem examination showed enlargement and hemorrhage of the adrenal glands. The other three females were killed *in extremis* due to findings of dystocia. Forestomach ulceration and/or adrenal cortical hypertrophy were observed in these three females.

At the 7.5 mg/kg/day dose, one female was found dead, and necropsy revealed evidence of dosing trauma as a possible cause of death. There were no male deaths at this dose.

At the 1mg/kg dose level, there was one male animal and two female animals killed *in extremis*. Necropsy showed respiratory problems as a possible cause of death in the male animal, and the cause of death in the two female animals could not be definitively determined.

In control animals, one male rat was killed during the first week of the study and one female rat was found dead during gestation. The male rat was killed due to accidental trauma that occurred to this animal, and the cause of death in the female rat could not be definitively determined.

In the F1 generation, mortality at the 15 mg/kg dose level was observed in a total of 10 males and five females. Death was attributed to the method of dosing and small size of the animals (as most deaths occurred during the maturation phase) based on necropsy results.

At the 7.5 mg/kg dose level, a total of four deaths occurred in male animals (three found dead, one killed in extremis). Death was attributed to dosing method and small animal size.

At the 1 mg/kg dose level, one male was killed in extremis and one male was found dead. The killed male was found to have a malignant lymphoma, and the found dead male was thought to have choked to death. One female was found dead at the 1 mg/kg dose level during the maturation phase, and was attributed to dosing error.
In control F1 animals, two males and three females were found dead or killed in extremis, and cause of death was dosing trauma except for one female, where the cause was attributed to dystocia.

The overall impression of the mortality in this study is one based on technical errors or causes unrelated to treatment.

The following clinical signs were observed in this study:

In P generation males, there were no significant clinical signs of toxicity at any of the dose levels tested in this study. In F1 males, there was a slight increase in the incidence of labored breathing and piloerection at the 15 mg/kg dose (3 rats each with these signs vs. 1 each in the control group).

In P generation females there was also a slight increase in the incidence of labored breathing and lethargy at the 15 mg/kg dose level (2 rats each with these signs vs. 0 rats in control).

There were no other observable clinical signs in P or F1 parental rats in this study at any dose level tested.

2. Body weight and food consumption: Data on body weight effects in this study for P and F1 generation adults was presented in Tables 4 and 5, pages 82-87 of the report, and Tables 14-15, pages 104-107 of the report. The data presented indicated no significant effect of treatment on body weight in either male or female rats during pre-mating, mating, gestation, or lactation.

Reported body weight and selected food consumption results are summarized in Table 4.

TABLE 4 Body Weight and Food Consumption - Pre-mating[a]

Observations/study week	Dose Group (mg/kg/day)			
	Control	1	7.5	15
P Generation Males - Pre-mating				
Mean body weight (g) Week 1	207± 9.7	208± 11.3	207± 9.9	209± 10.6

Mean body weight (g) Week 10	507± 42.4	510± 51.8	498± 34.9	500± 48.8
Mean weight gain (g) Weeks 1-10	300	302	291	291
Mean food efficiency Week 1	0.26	0.27	0.27	0.27
Mean food efficiency Week 10	0.07	0.07	0.06	0.06
P Generation Females - Pre-mating				
Mean body weight (g) Week 1	160± 8.9	158± 7.3	158± 8.8	157± 9.1
Mean body weight (g) Week 10	290± 22.2	287± 20.0	286± 23.3	283± 24.0
Mean weight gain (g) Weeks 1-10	135	134	134	134
Mean food efficiency Week 1	0.14	0.16	0.16	0.15
Mean food efficiency Week 10	0.03	0.03	0.04	0.05

a Data extracted from pages 82-87, 92, 94, and 104-107 of the report.
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

Data on group mean body weights and food consumption values for pregnant or nursing dams of both the P and F1 generations were shown in the report in Tables 14, 15, and 16, pages 104-109 of the report. These data showed no effect of treatment on either body weight or food consumption in females of either generation during gestation or lactation.

3. Test Substance Intake: The test substance was administered via gavage in this study. Verification of test article concentration over the course of the study was provided in the report (pages 655-658). At each time point measured (days 1, 4, 8, 15, 22, 57, 87, 120, 148, 176, 211, 239, and 260), the concentration found at each dose level did not deviate significantly from nominal concentration. As a percent of nominal concentration, the range for the 1.0 mg/kg dose level was 90-108%, 91-110% for the 7.5 mg/kg/day dose level, and 92-112% of nominal for the 15 mg/kg/day dose level.

4. Reproductive function:

a. Estrous cycle length and periodicity: A summary of estrous cycle measurements was presented in Table 10, pages 96-97 of the report for the P and F1 generations. Individual animal data were also provided in Appendix VIII, pages 272-287 of the report.

There did not appear to be any effect of treatment on estrous cycling in P or F1 female rats at any of the dose levels tested. However, the individual animal data did not present estrous cycling data for each of the animals over the 14 day measurement period, but only a single value for each animal. In addition, estrous cycling was not measured during cohabitation as specified in the 870.3800 guideline, but was measured only prior to cohabitation. So, it is uncertain whether any abnormalities were missed. The data suggest no obvious disturbance in estrous cycling for either the P or F1 generations; most had a normal 4/5 day cycle. .

b. Sperm measures: A summary of sperm measurements was presented in Tables 26 and 27, pages 131-132 of the report. In both the P and F1 generations, there were no effects of treatment on sperm concentration, sperm motility, or sperm morphology at any of the dose levels tested.

c. Sexual maturation (F_1): Summary data on sexual development were presented in Table 23, page 122 pf the report. These data present the group mean day of completion of sexual development, which for males means spearation of the prepuce from the glans penis, and for females, appearance of the vaginal opening. This is not specifically stated as such in the table but is interpreted to mean this. In the F1 offspring, treatment with THPS had no effect on the day post-partum on which sexual development was considered complete for males or females. The average days to sexual development completion was approximately 45 days for males and 41 days for females, with no significant differences among the dose groups.

5. Reproductive performance: Results for the parental animals are summarized from the report in Table 5.

TABLE 5 Reproductive Performance[a]

Observation	Dose Group (mg/kg/day)			
	Control	1.0	7.5	15.0
P Generation				
Mean precoital interval (days)	4	4	4	4
MALES				
Mated	31	32	31	32
Mating Index (%)	100	100	100	100
Fertility not determined	N/A			
Intercurrent deaths	0	0	0	0
FEMALES				
Number mated	32	32	31	32
Number fertile	31	32	31	29
Fertility not determined	0	0	1	2
Intercurrent deaths	0	1	0	2
Median gestation interval (days)	22	23	22	22
Number of litters	30	31	31	26

a Data extracted from (Appendix VIII, pages 272-279)
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

Total litter loss was observed in both control and dosed female P and F1 animals in the following incidence: Female P animals, incidence of 6/31, 2/32, 7/31, and 7/32; Female F1 animals, 1/32, 3/32, 0/32, and 2/32. This distribution of total litter loss does not indicate a specific effect of treatment with the test chemical.

5. Parental postmortem results

a) Organ weights: For the P generation males, absolute adrenal gland weight was decreased from control at the 7.5 and 15 mg/kg/day dose levels by 12% and 21% respectively ($p < 0.05$ and $p < 0.001$). Relative weight of the adrenal was also decreased in males at the 15 mg/kg/day dose level ($p < 0.01$). Testes weight was slightly decreased at the 15 mg/kg/day dose level (6% from control, $p < 0.05$).

In P generation females, relative but not absolute liver weight was increased by 12% over control at the 15 mg/kg/day dose level ($p < 0.01$). No other organ weight changes were noted in P generation females.

In F1 generation males, a significant increase in relative liver weight was again noted at the 15 mg/kg/day dose level (11% increase over control, $p > 0.001$). An increase in relative kidney weight was also noted at the 15 mg/kg/day dose (11% increase over control, $p < 0.01$), and a slight dose-response could be discerned (relative weights of 0.6582, 0.6695, 0.69890, and 0.7270 for the 0, 1.0, 7.5, and 15.0 mg/kg/day dose levels respectively).

In F1 female rats, a significant increase in both absolute and relative liver weight was noted at the 15 mg/kg/day dose level (increase of 10% and 12% respectively, $p < 0.01$ and 0.001). There were no other organ weight changes of note in F1 female rats.

b) Pathology

1) Macroscopic examination: Macroscopic post-mortem observations were presented in the report in Appendices XX and XXI, pages 456-471. There were no indications of any treatment-related effects from macroscopic examination of P or F1 generation parental animals.

2) Microscopic examination: In parental males and females of the P and F1 generation, periportal hepatocyte enlargement, periportal hepatocyte vacuolation, bile duct proliferation, and focal hepatocyte necrosis were observed in increased incidence at the 7.5 and 15 mg/kg/day dose levels of THPS.

The summary of the incidence of these findings is shown below.

Observation	Dose Group (mg/kg/day)			
	Control	1.0	7.5	15.0
P Generation				
MALES				
peripartal hepatocyte enlargement -minimal	0/31	0/32	18/31	24/32
periportal hepatocyte enlargement -slight	0/31	0/32	1/31	6/32
periportal hepatocyte vacuolation -minimal	0/31	0/32	22/31	5/32
periportal hepatocyte vacuolation -slight	0/31	0/32	0/31	24/32
periportal hepatocyte vacuolation - moderate	0/31	0/32	0/31	3/32
bile duct proliferation - minimal	1/31	0/32	3/31	11/32
bile duct proliferation - slight	0/31	0/32	0/31	3/32
focal hepatocyte necrosis present	1/31	0/32	3/31	6/32
periportal hepatocyte vacuolation -slight	0/31	0/32	0/31	24/32
FEMALES				
peripartal hepatocyte enlargement -minimal	5/32	6/32	68/32	16/32
periportal hepatocyte enlargement -slight	1/32	0/32	2/32	5/32
periportal hepatocyte vacuolation -minimal	2/32	4/32	13/32	11/32
periportal hepatocyte vacuolation -slight	0/32	0/32	1/32	13/32
periportal hepatocyte vacuolation - moderate	0/32	0/32	0/32	2/32
bile duct proliferation - minimal	0/32	1/32	0/32	0/32
focal hepatocyte necrosis present	0 /32	0/32	0/32	2/32

B. OFFSPRING

1. Viability and clinical signs: Clinical observations in offspring were presented in Appendix III of the report, pages 197-207 for the P-F1 generation. In the control group, there were reports of litter clinical signs in 13 litters. Of these 13 litters, total litter loss was reported as a clinical sign in 6 of these. The litters that were totally lost reported small appearance and no milk in the stomach, while the others reported tip of tail missing and/or small appearance. At the 1.0 mg/kg/day dose, 6 litters were reported with clinical signs, which included scattering of the litter about the cage, cold, weak, and no milk in the stomach. Two of the litters were total litter losses. At the 7.5 mg/kg/day dose, there were a total of 6 total litter losses and a total of 14 litters reporting clinical signs. As stated, 6 of these 14 were reported as total losses. Cold and weak appearance were associated with the total litter losses, while a small appearance, scattering about the cage, and thin fur was associated with most of the other 8 litters. At the 15.0 mg/kg/day dose, a total of 12 litters were reported with clinical signs, with a total of 8 reported as total litter losses. The total litter losses reported the same clinical signs as observed at lower doses, i.e. small, cold, no milk in stomach. The other litters reported cannabilization and/or small appearance.

   In the F1-F2 offspring, clinical signs reported were similar to those reported for the P-F1 generation and there did not appear to be an effect of treatment on the type or incidence of clinical signs reported.

   Mean litter size and viability results from pups from the P-F1 generation are summarized from the report in Table 7.

TABLE 7a Mean Litter Size and Viability[a]

Observation	Dose Group (mg/kg/day)			
	Control	1.0	7.5	15.0
P-F1 Generation Pups				
Mean litter size				
Day 1	12.3	13.2	13.0	11.3
Day 4	11.1	12.3	12.5	9.9
Day 7	7.3	8.0	7.8	7.6
Day 14	7.3	7.8	7.8	7.6
Day 21	7.3	7.8	7.8	7.6
Number live pups				
Day 0	391	428	430	319
Day 1	363	408	406	282
Day 4 (pre cull)	267	345	300	198
Day 14	176	217	187	136
Day 21	173	216	186	135
Number deaths				
Days 1-4	26	19	9	15
Days 4-21	3	1	1	1
Survival indices				
Viability index	74.8	84.6	77.1	70.2
Lactation index				

a Data extracted from pages 112-113, 352-359, 432-439
b After standardization (culling)

As some of the data for the above table were derived from the individual animal data tables in the study, there was no statistical analysis. Examination of the data above show slightly reduced mean litter size at the 15 mg/kg/day dose level and reduced number of live pups at this dose. Pup deaths, however, were not increased over lower doses or control. Of the total pup deaths between days 1-4, 84 of 96 deaths in the control were due to total litter losses, 30 of 63 deaths at the 1.0 mg/kg dose due to total litter losses, 102 of 106 deaths at the 7.5 mg/kg dose due to total litter losses, and 69 of 84 deaths due to total litter losses at the 15 mg/kg dose level. These are excluded from the pup death totals noted above.

Observation	Dose Group (mg/kg/day)			
	Control	1.0	7.5	15.0
F1-F2 Generation Pups				
Mean litter size				
Day 1	13.4	12.3	12.6	11.8
Day 4	13.2	12.2	12.5	11.4
Day 7	7.6	8.0	8.0	7.7
Day 14	7.4	8.0	7.9	7.7
Day 21	7.5	8.0	7.9	7.7
Number live pups				
Day 0	327	331	411	317
Day 1	321	308	392	296
Day 4 (pre-cull)	303	269	386	264
Day 14	173	239	239	196
Day 21	173	238	238	196
Number deaths				
Days 1-4	5	6	6	7
Days 4-21	0	1	1	0
Survival indices				
Viability index	74.8	84.6	77.1	70.2
Lactation index				

As with the P-F1 generation pups, there did not appear to be any significant effect of treatment with THPS on mean litter size, number of live pups, or number of pup deaths. There were no total litter losses in the control group of F1-F2 pups, 3 total litter losses at the 1.0 mg/kg dose level (33 pups total), no total litter losses at 7.5 mg/kg/day, and 2 total litter losses at 15 mg/kg/day (24 pups total).

2. Selected mean pup body weight data are presented in Table 8.

TABLE 8a Mean Litter Weight[a]

Day of lactation	Dose Group (mg/kg/day)			
	Control	1.0	7.5	15
P-F1 Generation				
Day 1	73.4	79.8	75.8	69.1
Day 4[b]	92.9	108.4	102.8	94.2
Day 7	102.3	115.6*	107.9	117.0*
Day 14	223.8	253.2	244.7	248.9
Day 21	366.3	416.2	399.6	408.2

a Data extracted from pages 114 of the report.
b unknown if weight obtained before or after culling
* Statistically different from control, $p<0.05$

TABLE 8b Mean Litter Weight[a]

Day of lactation	Dose Group (mg/kg/day)			
	Control	1.0	7.5	15
F1-F2 Generation				
Day 1	83.8	78.4	80.6	75.8
Day 4[b]	114.3	113.3	111.7	106.9
Day 7	111.4	122.6	117.9	118.0
Day 14	241.6	265.4	256.4	256.2
Day 21	390.2	428.5	408.9	409.8

a Data extracted from pages 115 of the report.
b unknown if weight obtained before or after culling
* Statistically different from control, $p<0.05$

There were no significant reductions in group mean litter weights for the P-F1 or F1-F2 generation at any dose level of THPS administered.

3 Offspring postmortem results: (pages 486-493)

a) Organ weights: not performed in this study. This is considered a deficiency. Section 7(c)(ii) of the 870.3800 guidelines states that "For F1 and F2 weanlings that are examined macroscopically, the [brain and spleen and thymus] should be weighed from one

randomly selected pup per sex per litter."

b) Pathology

1) Macroscopic examination: only macroscopic examination appears to have been performed on the pups in this study. According to the data in the study, the number of pups reported with macroscopic abnormalities and the types of abnormalities reported did not differ significantly among control and dosed pups.

2) Microscopic examination: There was no apparent microscopic examination of pups in this study. This is a minor deficiency as it is up to the study director to decide if the abnormalities observed at macroscopic examination are worthy of microscopic examination.

4. Offspring Reflexological Assessment (pages 120-121 of the report).

The assessment of reflexological responses of the offspring (surface righting reflex on day 1 post-partum, mid-air righting reflex on day 17 post-partum, startle reflex on day 21 post-partum, pupil reflex on day 21 post-partum) showed only one apparent effect, and that was noted by the reviewer as a decreased percentage of offspring in the F1-F2 generation at 15 mg/kg/day THPS that successfully responded to the surface righting reflex (78.7% vs. 90.9% in control, 81.9% at 1.0 mg/kg/day, and 91.4% at 7.5 mg/kg/day). However, there did not appear to be a dose-response relationship to this as the percentage was also decreased at the low dose but not the mid dose.

III. DISCUSSION

A. CONCLUSIONS:

The submitted study examined the effect of THPS on reproduction and fertility in rats at dose levels of 0, 1.0, 7.5, and 15.0 mg/kg over two generations. In parental animals, there were no significant effects of test article administration on body weight, food consumption, clinical signs, estrous cycling, or sperm parameters in both generations. Organ weights were affected at the 15 mg/kg/day dose level, and included an increase in liver weight for P generation females and F1 generation males and females. The increase in liver weight corresponded with histopathological alterations at the 15 mg/kg/day dose level, which included periportal hepatocyte enlargement, periportal

hepatocyte vacuolation, bile duct proliferation, and focal hepatocyte necrosis. These effects were observed also at the 7.5 mg/kg/day dose and were considered treatment-related, as there was a dose-response between the 7.5 and 15.0 mg/kg/day dose levels.
Reproductive performance and fertility were not significantly affected in parental animals of either generation. Although viability index was in the range of 70-80%, this value is not atypical for the rat.

Offspring measurements were also not significantly affected by treatment with THPS at any dose level, including litter size, pup weight, reflexological assessment, and macroscopic pathology. Organ weights of the brain and spleen and thymus, which are required by the 870.3800 guideline, were not provided in this report, so it is unknown what effect if any THPS may have had on these organ weights. As THPS appears to target the liver, it is of interest to examine offspring liver for any such effects.

Based upon the results of this study, the parental systemic toxicity NOAEL is determined to be 1 mg/kg/day, based upon the histological alterations of the liver observed in both parental generations at the 7.5 mg/kg/day dose. The reproductive toxicity NOAEL is provisionally determined to be $\geq$ 15 mg/kg/day, and the reproductive toxicity LOAEL is provisionally determined to be > 15 mg/kg/day, as there were no significant effects observed in offspring at any dose level tested in this study.
Histopathological assessment of selected pup organs was claimed in the summary of the report, but no results were presented.

C. STUDY DEFICIENCIES: Required organ weights were not obtained in the offspring in this study and the histopathology that was stated to have been performed in the offspring was not included in the results section or in the data tables of the report. Also, estrous cycling was not measured during cohabitation. Offspring organ weights should be provided if available and, histopathology data should be submitted as these data are considered of importance in determining the effects of treatment on the offspring.